WE CLAIM:

1. A polypeptide having lipase activity; wherein said polypeptide is a triacylglycerol hydrolysing enzyme; and wherein said polypeptide is capable of hydrolysing glycolipids that are normally present in a flour to the corresponding galactosyl monoglycerides, wherein said polypeptide is capable of hydrolysing at least 10% of galactosyl diglycerides normally present in a flour dough to monoglycerides.

2. A polypeptide having lipase activity; wherein said polypeptide is a triacylglycerol hydrolysing enzyme and wherein said polypeptide retains at least 82.5% activity after 4 days at room temperature and at a pH in the range of 3.5-8, and wherein said polypeptide is capable of hydrolysing glycolipids that are normally present in a flour to the corresponding galactosyl monoglycerides, wherein said polypeptide is capable of hydrolysing at least 10% of galactosyl diglycerides normally present in a flour dough to monoglycerides.

3. A polypeptide having lipase activity; wherein said polypeptide is a triacylglycerol hydrolysing enzyme, wherein said polypeptide retains at least 82.5% activity after 4 days at room temperature and at a pH in the range of 3.5-8.

4. A polypeptide having lipase activity; wherein said polypeptide is a triacylglycerol hydrolysing enzyme; and wherein said polypeptide is capable of hydrolysing glycolipids, monogalactosyl diglyceride and digalactosyl diglyceride, that are normally present in a flour to monogalactosyl monoglyceride and digalactosyl monoglyceride.

5. A polypeptide having lipase activity; wherein said polypeptide is a triacylglycerol hydrolysing enzyme and

wherein said polypeptide retains at least 82.5% activity after 4 days at room temperature and at a pH in the range of 3.5-8, and wherein said polypeptide is capable of hydrolysing glycolipids, monogalactosyl diglyceride and digalactosyl diglyceride, that are normally present in a flour, to the corresponding galactosyl monoglycerides.

6. A polypeptide having lipase activity; wherein said polypeptide is a triacylglycerol hydrolysing enzyme and wherein said polypeptide is capable of modifying by hydrolysis the glycolipids, monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) to the more polar components monogalactosyl monoglyceride (MGMG) and digalactosyl monoglyceride (DGMG).

7. A polypeptide having lipase activity; wherein said polypeptide is a triacylglycerol hydrolysing enzyme and wherein said polypeptide retains at least 82.5% activity after 4 days at 20°C and at a pH in the range of 3.5-8, wherein said polypeptide is capable of modifying by hydrolysis the glycolipids, monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG), to the more polar components monogalactosyl monoglyceride (MGMG) and digalactosyl monoglyceride (DGMG).

8. A polypeptide having lipase activity; wherein said polypeptide is a triacylglycerol hydrolysing enzyme and wherein said polypeptide is capable of hydrolysing glycolipids that are normally present in a flour to galactosyl monoglycerides, wherein said polypeptide is capable of modifying by hydrolysis the glycolipids, monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) to the more polar components monogalactosyl monoglyceride (MGMG) and digalactosyl monoglyceride (DGMG).

9. A polypeptide having lipase activity; wherein said polypeptide is a triacylglycerol hydrolysing enzyme and

wherein said polypeptide is capable of hydrolysing galactosyl diglycerides that are normally present in a flour to galactosyl monoglycerides, wherein said polypeptide is capable of hydrolysing at least 10% of the galactosyl diglycerides normally present in a flour dough to monoglycerides, wherein said polypeptide is capable of modifying by hydrolysis the galactosyl diglycerides, monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG), to the more polar components monogalactosyl monoglyceride (MGMG) and digalactosyl monoglyceride (DGMG).

10. A polypeptide having lipase activity; wherein said polypeptide is a triacylglycerol hydrolysing enzyme and wherein said polypeptide retains at least 82.5% activity after 4 days at 20°C and at a pH in the range of 3.5-8, and wherein said polypeptide is capable of hydrolysing glycolipids that are normally present in a flour to galactosyl monoglycerides, wherein said polypeptide is capable of modifying by hydrolysis the glycolipids, monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) to the more polar components monogalactosyl monoglyceride (MGMG) and digalactosyl monoglyceride (DGMG).

11. A polypeptide having lipase activity; wherein said polypeptide is a triacylglycerol hydrolysing enzyme and wherein said polypeptide retains at least 82.5% activity after 4 days at 20°C and at a pH in the range of 3.5-8, and wherein said polypeptide is capable of hydrolysing galactosyl diglycerides that are normally present in a flour to the corresponding galactosyl monoglycerides, wherein said polypeptide is capable of hydrolysing at least 10% of the galactosyl diglycerides normally present in a flour dough to the monoglycerides, wherein said polypeptide is capable of modifying by hydrolysis the galactosyl diglycerides, monogalactosyl diglyceride (MGDG) and digalactosyl digly-

ceride (DGDG) to the more polar components monogalactosyl monoglyceride (MGMG) and digalactosyl monoglyceride (DGMG).

12. A polypeptide having lipase activity; wherein said polypeptide is capable of modifying by hydrolysis the glycolipids, monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) to the more polar components monogalactosyl monoglyceride (MGMG) and digalactosyl monoglyceride (DGMG).

13. A polypeptide comprising at least one amino acid sequence shown herein as SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3.

14. A polypeptide according to claim 1 wherein the polypeptide is derivable from *Aspergillus tubigensis*.

15. A polypeptide according to claim 1, wherein the polypeptide has the following characteristics:

(i) it retains at least 80% activity after 4 days at 20°C at a pH in the range of 3.5-8,

(ii) it retains at least 60% of its activity after 1 hour at 60°C in 100 mM sodium acetate buffer at pH 5.0, and

(iii) it has an isoelectric point as determined by isoelectric focusing of 4.1±0.1.

16. A polypeptide according to claim 1 wherein said polypeptide comprises at least one amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3, wherein Xaa in said sequences is an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val.

17. A polypeptide according to claim 1 wherein said lipase has an enzymatic activity at a pH in the range of 3.5-8.

18. A polypeptide according to claim 1, which polypeptide retains at least 80% of its activity after 1 hour at 50°C in 100 mM sodium acetate buffer at pH 5.0.

19. A polypeptide according to claim 1 which polypeptide has an isoelectric point as determined by isoelectric focusing of 4.1±0.1.

20. A polypeptide according to claim 1 that is capable of hydrolysing at least 10% of the galactosyl diglycerides normally present in a flour dough to the corresponding galactosyl monoglycerides.

21. A polypeptide according to claim 1 wherein the polypeptide is in a substantially purified form.

22. A polypeptide according to claim 1 wherein the polypeptide has a molecular weight as determined by matrix-assisted laser desorption ionisation mass spectrometry (MALDI-MS) of 31 ± 1.5 kDa.

23. A polypeptide according to claim 1 wherein the polypeptide comprises the amino acid sequence shown as SEQ ID NO:9 or a variant, homologue or fragment thereof.

24. A polypeptide according to claim 1 wherein the polypeptide is derived from an organism including a fungus, a yeast, a bacterium, a plant cell or an animal cell.

25. A polypeptide according to claim 1 wherein when the polypeptide is added to a bread dough in an amount of 5,000 LUS per kg flour it reduces the average pore diameter of the crumb of the bread made from the dough by at least 10%, relative to a bread which is made from a bread dough without addition of the polypeptide.

26. A polypeptide according to claim 1 wherein when it is added to a bread dough in an amount of 5,000 LUS per kg flour, it increases the pore homogeneity of the crumb of the bread made from the dough by at least 5%, relative to a bread which is made from a bread dough without addition of the polypeptide.

27. A polypeptide according to claim 1 wherein when it is added to a bread dough in an amount of 5,000 LUS per kg flour, it increases the gluten index in the dough by at least 5%, relative to a dough without addition of the polypeptide, the gluten index being determined by means of a Glutomatic 2200 apparatus.

28. A recombinant DNA molecule comprising a nucleotide sequence coding for the polypeptide according to claim 1.

29. A recombinant DNA molecule according to claim 28 comprising at least one of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7.

30. A recombinant DNA molecule according to claim 28 comprising SEQ ID NO:8 or a variant, homologue or fragment thereof or a sequence complementary thereto.

31. A recombinant DNA molecule according to claim 28 which is a plasmid deposited under the accession No. NCIMB 40863.

32. A cell comprising a recombinant DNA molecule according to claim 28 and capable of expressing the polypeptide according to claim 1.

33. A cell according to claim 32 which is a microorganism comprising a fungus, a yeast, a bacterium, a plant cell or an animal cell.

34. A cell according to claim 33 which is a filamentous fungus comprising an *Aspergillus* sp., a *Penicillium* sp., a *Rhizomucor* sp., or a *Neurospora* sp.

35. A cell according to claim 34 which is *Aspergillus tubigensis*.

36. A method of preparing a polypeptide according to claim 1 comprising transforming a host cell with a recombinant DNA molecule according to claim 28, the host cell being capable of expressing the nucleotide sequence coding for the polypeptide, cultivating the transformed host cell under conditions where the nucleotide sequence is expressed and harvesting the polypeptide.

37. A method according to claim 36 which comprises a further step of isolating the polypeptide in a substantially pure form.

38. A method of preparing a baked product having improved pore homogeneity and reduced average pore diameter, the method comprising adding the polypeptide according to claim 1.

39. A method according to claim 38 wherein the dough does not contain added lipids.

40. A method according to claim 38, comprising adding to the dough the polypeptide in an amount that results in a reduction of the average pore diameter in the crumb of the bread made from the dough by at least 10%, relative to a bread which is made from a bread dough without addition of the polypeptide.

41. A method according to claim 38, comprising adding to the dough the polypeptide in an amount that results in an increase of the pore homogeneity in the crumb of the bread made from the dough by at least 5%, relative to a bread

21. ☒ A check in the amount of **$ 2,550.00** is enclosed. In the event any variance exists between the amount enclosed and the Patent Office charges, please charge or credit any such variance to **Deposit Account No. 50-0206**.

☒ The Commissioner is hereby authorized to charge any variance between the amount enclosed and the Patent Office charges to **Deposit Account No. 50-0206**.

Respectfully submitted,

By: _____
Stanislaus Aksman
Registration No. 28,562

SA/cvc
Enclosures

which is made from a bread dough without addition of the polypeptide.

42. A method according to claim 38, comprising adding to the dough the polypeptide in an amount that results in an increase of the gluten index in the dough of at least 5%, relative to a dough without addition of the polypeptide, the gluten index being determined by means of a Glutomatic 2200 apparatus.

43. A method according to claim 38 wherein the polypeptide is added to the dough in an amount which is in the range of 5,000-30,000 lipase units (LUS) per kg flour.

44. A method according to claim 38 wherein an emulsifier is added to the dough.

45. A method of improving the stability of a gluten network in a dough, imparting improved pore homogeneity, reducing pore diameter of a baked product made from the dough or a combination thereof, comprising adding to the dough a polypeptide according to claim 1 or a polypeptide prepared by a process according to claim 36.

46. A method according to claim 45 wherein the gluten index in the dough is increased by at least 5%, relative to a dough which is made without addition of the polypeptide, according to claim 1 or the polypeptide prepared by a process according to claim 36, the gluten index being determined by means of a Glutomatic 2200 apparatus.

47. A dough improving composition comprising the polypeptide according to claim 1 and at least one further conventional dough additive component.

48. A recombinant DNA molecule comprising a nucleotide sequence coding for a polypeptide exhibiting lipase activity and which polypeptide comprises at least one of the amino

acid sequences shown herein as SEQ ID NO:1, SEQ ID No:2 and SEQ ID NO:3 or a nucleotide sequence coding for a polypeptide exhibiting lipase activity which comprises the amino acid sequence shown as SEQ ID No. 9.

49. A recombinant DNA molecule comprising at least one of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and SEQ ID NO:7 or at least the coding sequence of the nucleotide sequence shown as SEQ ID NO:8 or a variant, homologue or fragment thereof, or a sequence complementary thereto.